

Serial No.: 09/121,239
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Group Art Unit: 1635

Amendment Accompanying RCE
Docket No. GP091-03.RC1

at least one nucleic acid polymerase activity;

SUB
D1
c) amplifying the fusion nucleic acid in an isothermal nucleic acid amplification reaction using the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the first single-stranded fusion nucleic acid that contains the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to sequence complementary to the first primer binding site;

RE
d) hybridizing the second nucleic acid strands with an oligonucleotide probe under hybridization conditions in which the probe hybridizes to either the first probe binding site or the second probe binding site, thereby forming a probe:target hybrid; and

e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample.

SUB
D4
6. (Amended Twice) The method of Claim 1, wherein step a) further comprises preparing the sample containing the fusion nucleic acid by:

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contacting a biological sample comprising the fusion nucleic acid with a solution [comprising]
consisting essentially of:

a buffer,

about 150 mM to about 1 M of a soluble salt,

about 0.5% to about 1.5% [(v/v)] (v/v) of a non-ionic detergent, and

a solid support to which is joined an immobilized oligonucleotide comprising a

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SUB D⁴
nucleotide base sequence which forms, directly or indirectly, a stable hybridization complex with an RNA under conditions permitting the formation of the stable hybridization complex; and

separating the hybridization complex joined to the solid support from unhybridized sample components.

9. (Amended Twice) A method of detecting a fusion mRNA transcript produced as a result of a chromosomal translocation, comprising:

SUB D⁵
a) providing a sample containing a fusion mRNA transcript comprising a splice junction;

b) contacting under isothermal nucleic acid amplification conditions:

the fusion mRNA transcript,

a first primer which hybridizes to the fusion mRNA transcript at a first primer binding site derived from a first chromosomal region and located 3' to the splice junction site, and

at least one enzyme having nucleic acid polymerase activity;

AZ
c) amplifying the fusion mRNA transcript in a nucleic acid amplification reaction that uses the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the fusion mRNA transcript containing the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, wherein the first probe binding site is derived from a second chromosomal region, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site is derived from a third

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D₅ (cont.)
- chromosomal region and overlaps or is located 3' to sequence complementary to the first primer binding site;
- d) hybridizing the second nucleic acid strands with an oligonucleotide probe which hybridizes to the second nucleic acid strands at either the first probe binding site or the second probe binding site but does not hybridize to the fusion transcript, thereby forming a hybridization complex of the probe and the second nucleic acid strand; and
- e) detecting the hybridization complex as an indication of the presence of the fusion transcript in the sample.

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D₉
19. (Amended Twice) A method of preparing a sample containing RNA suitable for amplification, comprising the steps of:
- a) providing a biological sample comprising unpurified RNA;
- b) mixing the biological sample with a solution [comprising] consisting essentially of:
a buffer at a pH of about 6.5 to about 8.5,
[at least] about 150 mM to about 1 M of a soluble salt,
[an effective amount] about 0.5% to about 1.5% (v/v) of a non-ionic detergent
[sufficient to release RNA from the biological sample without causing viscosity due to release of chromosomal DNA], and with
a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions;
- c) separating the hybridization complex joined to the solid support from unhybridized sample components; and
- d) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex.